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Growth promoting activity of *Pangasianodon hypophthalmus* recombinant growth hormone expressed in *Escherichia coli*

MEGARAJAN SEKAR[1], SHIVA DHAR SINGH[2], RAYMOND JANI ANGEL[3], DHARMENDRA KUMAR MEENA[4], NACHIMUTHU SIVAKUMAR[3], ESWARAN SURESH[3] AND A. KATHIRVELPANDIAN[5]

[1]*Regional Centre of Central Marine Fisheries Research Institute, Visakhapatnam - 530 003, Andhra Pradesh, India*
[2]*Indian Council of Agricultural Research (ICAR), KAB II, Pusa, New Delhi -110 012, India*
[3]*Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Andheri (W), Mumbai - 400 061, Maharashtra, India*
[4]*Central Inland Fisheries Research Institute, Barrackpore, Kolkata - 751 002, West Bengal, India*
[5]*National Bureau of Fish Genetic Resources Kochi Unit, Kochi - 682 018, Kerala, India*
e-mail: sekarrajaqua@gmail.com

ABSTRACT

Recombinant growth hormone of *Pangasianodon hypophthalmus* (rPhGH) was efficiently expressed in *Escherichia coli* BL 21 (DE3) cells. The expression vector pET-32a(+) was used to clone and express a 550 bp long cDNA fragment, which encodes the mature region of growth hormone. The rPhGH was expressed as a 6X HIS-tag fusion protein in *E. coli* upon induction by Isopropyl β-D-thiogalactoside, and formed insoluble inclusion bodies in the host cells. SDS-PAGE analysis indicated that the molecular weight of the fusion protein was about 23 kDa, which is comparable to the theoretical value of the mature growth hormone of the fish. The expressed protein was recovered by solubilising the inclusion bodies under denaturing conditions with urea and then the denatured proteins were refolded and purified on Ni-NTA column. The purified recombinant protein was confirmed by Western blot analysis using anti-His antibodies. Total yield of the refolded and purified protein was 20 mg l^{-1} of LB medium. Biological activity of the purified recombinant protein was determined in *in vivo* bioassay by its ability to promote growth in rohu (*Labeo rohita*) fingerlings, injected with three different concentrations of the hormone. A significant increase in growth was observed in rohu fingerlings administered with rPhGH at a dosage of 1.0 µg g body weight $^{-1}$.

Keywords: Aquaculture, Growth hormone, *Pangasianodon hypophthalmus,* Recombinant DNA technology

Introduction

Fish contributes to food security in many regions of the world, providing a valuable supplement for diversified and nutritious diets (FAO, 2011). Over half of the aquatic species consumed is mainly produced from aquaculture and an estimate of additional 40 million t of aquatic food will be required by 2030 to meet the demand of increasing human population. At present, aquaculture contributes around 40% of the world fish production (FAO, 2013). However, the production is constrained mainly by the growth efficiency of cultured fish species. Growth rates of many fish species used in aquaculture are naturally slow, but are being enhanced by methods of domestication, nutritional approach, genetic selection, biotechnological as well as endocrinological approaches (Delvin *et al*., 2001). Endocrine approaches in controlling growth have been explored extensively through various means, principally by applications of somatotropins such as growth hormone (GH), prolaction, placental lactogen, insulin like growth factor-1 (IGF-1), thyroid hormones, and sex steroids (McLean and Delvin, 2000). A significant level of growth improvement has been achieved in fishes through application of these growth related hormones.

Fish growth hormone (GH) is produced by anterior pituitary cells and is involved in the regulation of growth, development, metabolism, appetite and osmoregulation in fishes (Silverstein *et al*., 2000). It is widely used to study feed intake efficiency and growth in different fishes. Growth hormone therapy is commonly used to enhance growth in domestic animals and it has become increasingly popular in aquaculture for finfishes and shellfishes (Ayson *et al*., 2000; Funkenstein, 2006; Linan-Cabello *et al.,*

2013). Initially, mammalian growth hormone (GH) was found to accelerate the growth of fish and later, different approaches to accelerate growth by administration of recombinant fish GH were explored and encouraging results have been reported in different fish species such as flounder (Jeh *et al*., 1998), giant catfish (Promodonkoy *et al*., 2004) and rabbit fish (Funkenstein *et al.*, 2005).

Recombinant DNA technology has revolutionised the biological science and recent advances in this field provide the means of large scale production of various recombinant proteins for commercial applications. Due to their effect on growth promotion, food conversion efficiency, and other physiological processes such as reproduction and osmoregulation (Farmanfarmaian and Sun, 1999), recombinant GHs have been demonstrated to have potential in agricultural and aquacultural applications. The fish recombinant growth hormone has been successfully produced from a large variety of species in different expression systems and currently used in aquaculture industry. Among these are the common carp, Indian major carp, tilapia, tuna, barramundi, rainbow trout, turbot, seabass and eel (Funkenstein, 2006). *Pangasianodon hypophthalmus* (sutchi catfish) is one of the fast growing catfish and popular fish species in some of the Asian countries (Khan *et al.,* 2009). Its growth rate is influenced by many factors, among which the growth hormone may be one of the crucial factors playing role in fish growth similar to other vertebrate growth hormones (Promdonkoy *et al*., 2004). The present study was designed with an objective to produce recombinant growth hormone of *P. hypophthalmus* in *Escherichia coli* and to evaluate its biological activity in rohu (*Labeo rohita)*

Materials and methods

Experimental animal, bacterial strain, expression vector, template DNA and primers

Recombinant growth hormone of *P. hypophthalmus* (rPhGH) was produced and its biological activity was evaluated in 2 to 3 months old rohu (*Labeo rohita*) fingerlings, obtained from Kharland Research Station, Mumbai, India. *Escherichia coli* BL21 (DE3): F^{-}ompT gal dcm lon hsdS $_{B}$ (r_{B}^{-} m_{B}^{-}) λ (DE3 [lacl lacUV5-T7 gene I ind I sam7 nin5]) strain was used as host for expression of rPhGH. His-tag plasmid vector, pET-32a(+) (Novagen, USA) was selected as an expression vector for rPhGH expression. The recombinant plasmid containing the open reading frame (ORF) of *P. hypophthalmus* GH gene was constructed using pTZ57R/T cloning vector (Fermentas) and named as pTZ-PhGH. This gene construct was developed in our earlier experiment and the gene sequence of ORF region has been submitted in NCBI GenBank under the accession number GQ859589. The developed recombinant plasmid (pTZ-PhGH) was used as template for PCR amplification of cDNA fragment encoding the mature PhGH. The primer set used for amplification of cDNA encoding mature PhGH was designed, using the gene sequence in the cDNA sequences of PhGH gene (NCBI GenBank: GQ859589). The primer sequences designed are follows: forward: 5'-AAAGAATTCATTC GAGAACCAGCGGCTCTTC-3', and reverse: 5'-AA AAAGCTTCAGGGTGCAGTTGGAATCCAG-3' with *Eco*RI and *Hind*III restriction enzyme sites (underlined nucleotides) at 5' end, respectively.

Construction of recombinant expression vector for PhGH expression

The cDNA fragment encoding mature PhGH was obtained by PCR amplification with specific forward and reverse primers. The PCR reaction was performed for an initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 50s, annealing at 59°C for 60s and extension at 72°C for 2 min and then finally a single cycle of final extension at 72°C for 10 min. The amplified fragment was digested with *Eco*RI and *Hind*III restriction enzymes and then cloned into the same enzyme site in the multiple cloning sites of pET-32a(+) vector. The constructed plasmid was denominated with pET-PhGH and transformed into competent cells of *E. coli* BL21 (DE3) for expression of fusion protein. The transformed cells were selected on LB agar plates containing ampicillin (100 μg ml^{-1}) and chloramphenicol (25 μg ml^{-1}) and further the recombinant plasmid was reconfirmed by restriction enzyme analysis with *Eco*RI and *Hind*III. The positive colonies growing on the plates were selected and used for the expression.

Expression of recombinant PhGH and SDS-PAGE analysis

The transformed *E. coli* BL21 (DE3) cells containing pET-PhGH were grown overnight at 37°C in LB medium containing 100 μg ml^{-1} of ampicillin and 25 μg ml^{-1} of chloramphenicol. Fresh LB broth with 100 μg ml^{-1} of ampicillin was inoculated with the non-induced overnight culture. The culture was further grown at 37°C with vigorous shaking until the growth reached mid log phase (OD_{600} of 0.6) and was induced with 1 mM IPTG (Isopropyl β-D-thiogalactoside) (Sigma, USA). The induced culture was grown for another 5-6 h and harvested by centrifugation at 4000 *g* for 20 min at 4°C. The harvested cell pellet was washed with phosphate buffered saline (PBS) and dissolved in non-denaturing lysis buffer

(50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH. 8). The cells in the buffer were subjected to freeze thawing once and then centrifuged at 4000 *g* for 20 min at room temperature. The supernatant solution was collected and marked as soluble fraction and then the pellet containing insoluble fraction was resuspended in appropriate volume of 2X SDS-PAGE sample buffer (0.09 M Tris·Cl, pH 6.8; 20% glycerol; 2% SDS; 0.02% bromophenol blue; 0.1 M DTT), and boiled at 100°C for 5 min, then cell pellet lysate was collected after centrifugation. The supernatant of soluble fraction and cell pellet lysate were analysed for recombinant protein expression and its solubility on 12.5% SDS polyacrylamide gel electrophoresis (SDS-PAGE) along with the uninduced bacterial cell lysate as control.

Purification and refolding of recombinant PhGH

For recombinant protein purification, the induced cells were cultured in 250 ml flask and pelleted by centrifugation at 4000 *g* for 20 min, and then the pellet was resuspended in 8 ml of cell lysis buffer (100 mM NaH_2PO_4, 10 mM Tris-HCl, 8 M urea, pH 8). Cells in lysis buffer were lysed by sonication on ice, and the cell lysate was centrifuged at 6000 *g* for 20 min at room temperature to pellet the cell debris. The supernatant was separated and mixed with nickel-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen, Germany) at a ratio of 4:1, and then the mixture was vigorously mixed at 200 rpm for 1 h. The lysate-resin mixture was loaded into an empty column with attached bottom cap and incubated for 2 h at room temperature. Then, the column with protein was shifted to 16°C and the unbound protein was collected as flow through after removing the bottom cap. The Ni-NTA column based refolding and purification was performed as per procedure followed by Saini *et al*. (2002). In this, the column was washed with denaturing buffer containing 20 mM imidazole and then washed stepwise with decreasing concentration of urea (6 - 1 M) in refolding buffer (20 mM Tris HCl, pH 8.0, 500 mM NaCl, 10% glycerol, 20 mM imidazole, 0.5 mM oxidized glutathione (GSSG) and 5 mM reduced glutathione (GSH)). Then again, the column was washed with refolding buffer without urea to remove contaminating *E. coli* proteins. The bound protein was eluted with 5 ml of refolding buffer containing 250 mM of imidazole in fractions of 1 ml. All the fractions were analysed in SDS-PAGE, then the peak fractions were pooled and dialysed against storage buffer (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 50% glycerol, 0.1 mM DTT) and stored at 4 °C overnight. After dialysis, the protein was analysed in SDS-PAGE for its refolding and purity. Western blot analysis of purified protein was carried out by QIA express detection and assay kit (Qiagen, Germany) based on manufacturer's protocol. The protein was detected using HRP conjugated anti-histidine antibody (Qiagen, Germany). The concentration of purified and refolded recombinant GH was determined by Bicinchonic Acid (BCA) method with bovine serum albumin (BSA) as the standard using a protein estimation kit (Bangalore Genei, India). Standard was prepared as per the manufacturer's instructions.

Determining biological activity of recombinant PhGH

The growth promoting activity of purified and refolded rPhGH was tested *in vivo* with 2 to 3 months old rohu (*Labeo rohita*) fingerlings. A total of 240 fingerlings were randomly divided in to four different groups of 20 fishes per group, namely control and three treatments (T1, T2 and T3) with three replicates, and all the treatment groups were stocked in 500 l capacity FRP tanks. The rPhGH was diluted in biological saline (0.85% NaCl) and administered at dosages of 0.25, 0.5 and 1 μg g^{-1} body weight of fingerlings for T1, T2 and T3, respectively. The injections were given once in a week for four weeks, by intramuscular injection**.** The dosage was determined based on the data provided by previous studies (Silverstein *et al*., 2000). The entire experiment was carried out for 12 weeks and fishes in each group were weighed individually, once in a week for 12 weeks. During the experiment, all the fishes were fed with commercial feed twice a day at 5 to 7% of total body weight. Water quality was maintained within the safe limits for the species for all the groups and water exchange was done periodically as and when required.

Statistical analysis

One way analysis of variance (ANOVA) was performed using SPSS statistical software package (SPSS version 15, USA). All the data were subjected to one way ANOVA and Duncan's multiple range tests (Duncan, 1955) to determine the differences in means ($p \leq 0.05$). The mean of 20 observations per group with replicates was used for growth analysis. All parametric data are expressed as mean ± SE.

Results

Expression of recombinant PhGH in E. coli

The cDNA fragment encoding mature PhGH was PCR amplified from the pTZ-PhGH recombinant plasmid and was inserted into the pET-32a(+) expression vector to obtain the pET-PhGH (Fig. 1) plasmid construct with an aim of obtaining *E. coli* BL21 (DE3) strain producing

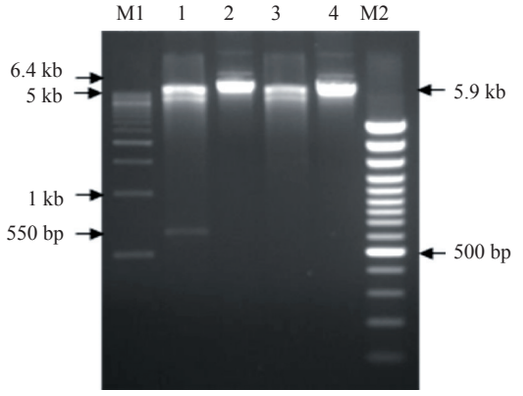

Fig 1. Restriction enzyme digestion of recombinant plasmid (pET PhGH) containing pET-32a expression vector and the gene encoding mature region of PhGH
Lane M1: Molecular weight marker (500 bp DNA ladder up to 5kb); Lane 1: The expected PhGH gene insert (550 bp) released after digestion with *Eco*RI & *Hind*III; Lane 2: Recombinant plasmid with PhGH gene insert (~6.4 kb); Lane 3: *Eco*RI & *Hind*III digest of control pET-32a plasmid (5.9 kb); Lane 4: Uncut control plasmid (5.9 kb); Lane M2: Molecular weight marker (100 bp) DNA ladder up to 3 kb

intracellular PhGH protein. Recombinant growth hormone was expressed in transformed *E. coli* BL21 culture after induction with 1 mM of IPTG. The expression of rPhGH was confirmed in SDS-PAGE by observing the presence of a distinct band corresponding to the estimated rPhGH size of about 23 kDa (Fig. 2a). The distinct band of particular size was found in cell lysate of induced *E. coli* cells containing recombinant plasmid. However, the same band was absent in uninduced transformed *E. coli* cells, which confirmed the expression of the recombinant protein after induction. Further, the solubility of the expressed protein was analysed in SDS-PAGE, in which the supernatant of cell lysis buffer was marked as soluble fraction and cell pellet lysate obtained after boiling was marked as insoluble fractions, and both the fractions were analysed on SDS-PAGE. The recombinant protein was found only in an insoluble fraction and was absent in soluble fraction. The protein solubility analysis revealed that the rPhGH was expressed at high levels but confined exclusively to the insoluble fraction as inclusion bodies.

Purification and matrix assisted refolding of PhGH

The recombinant fusion PhGH expressed as inclusion bodies was solubilised using sonication in cell lysis buffer containing strong denaturing agent (8M urea). SDS-PAGE analysis of the solubilised protein indicated that the protein was expressed along with several cellular proteins of *E. coli*, which is evidenced by the presence of many bands along with the targeted protein (Fig. 2a). The recombinant fusion PhGH was purified from *E. coli* under denaturing conditions using Ni-NTA column along with other several cellular proteins. The denatured fusion protein was simultaneously renatured using matrix assisted refolding method and purified from other cellular proteins. While purification, five different fractions were collected and analysed in SDS-PAGE (data not shown) and among all, the fractions 3-5 were identified as peak fractions. All the peak fractions of purified proteins were dialysed after pooling and a total of 10 ml of elute was recovered after dialysis. SDS-PAGE analysis of dialysis-purified protein showed a single band with the expected molecular weight of about 23 kDa (Fig. 2b). The presence

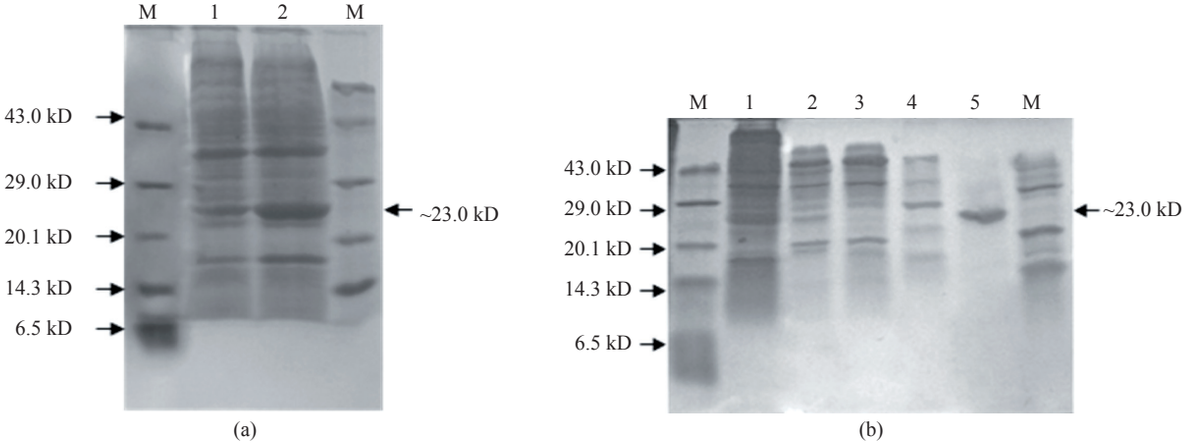

Fig. 2. Expression and purification of recombinant PhGH
(a) Expression of recombinant growth hormone of *P. hypophthalmus* in *E. coli*. Lane M: Protein molecular weight markers; Lane 1: Cell lysate from un-induced cells (control); Lane 2: Induced cells expressed recombinant PhGH with molecular weight of ~23.0 kDa
(b) Purification of recombinant PhGH by Ni-NTA agarose column. Lane M: Protein molecular weight markers; Lane 1: Cell lysate; Lanes 2-4: Wash; Lane 5: Elutes (Purified and refolded recombinant PhGH after dialysis).

of a single protein band confirmed that the protein was purified and properly refolded in the column. Western blot analysis of the purified protein from the 12.5% SDS-PAGE under reducing condition showed that the protein was immunoreactive to an anti-His antibody. The observed molecular weight of the protein was ~23 kDa (Fig. 3), which is identical to an estimated size of the protein and confirmed that the expressed and purified protein was PhGH. The concentration of the purified protein was estimated as 520 mg ml^{-1} and the estimated total concentration of purified protein was 20 mg l^{-1} from 250 ml of *E. coli* broth culture.

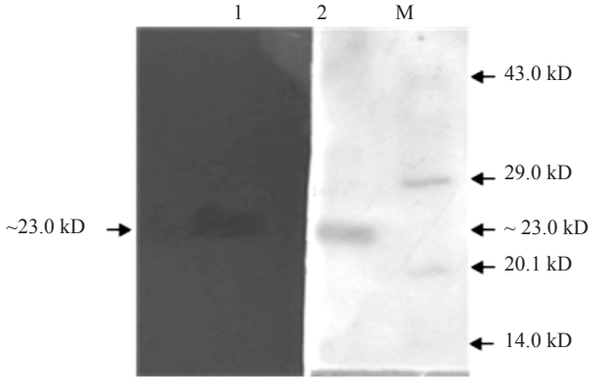

Fig. 3. Western blot analysis of purified recombinant growth hormone with anti His antibodies. Lane 1: Purified PhGH after western blot; Lane 2: Purified PhGH after SDS-PAGE (control); Lane M: Protein molecular weight markers

Determining biological activity of rPhGH

The biological activity of the recombinant PhGH was assessed in *in-vivo* bioassay by measuring the differential weight gain in the fishes injected with recombinant growth hormone and control. The average weight gain of fishes in control and treatments (T1, T2 and T3) were 4.7, 5.42, 7.35 and 9.39 g and the average initial and final weight were 14.42, 14.55, 13.92, 14.91 and 19.12, 19.97, 21.27, 24.30 g respectively (Fig. 4). Among the treatment groups, the maximum growth was observed for T3 administered with 1.0 µg g^{-1} body weight of the hormone. The mean, standard error and differences in weight gain for the weekly growth were calculated by one way ANOVA (Table 1). From the analysis, it is observed that treatment 3 (T3) showed significant differences in growth gain when compared to control, and other treatment (T1 and T2) groups from third week onwards. The weight gain between the T1 and T2 groups did not show any significant difference. However, these two treatments showed significant difference ($p \leq 0.05$) with control. The cumulative weight gain in the third month (4.8 g) was observed to be significantly high

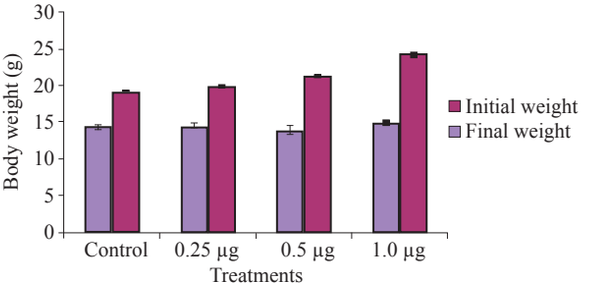

Fig. 4. Biological activity of PhGH. Graphical representation of mean ± standard error (n = 20) for initial and final average weight of the fish treated with different dosage of purified rPhGH per g body weight of *L. rohita*

Table 1. Biological activity of *Pangasianodon hypophthalmus* growth hormone (PhGH)

Treatments / Week	Control	T1	T2	T3
1	14.42±0.40	14.55±0.32	13.92±0.56	14.91±0.26
2	14.66±0.11	15.07±0.17	14.86±0.23	15.77±0.08
3	15.06^{a}±0.38	15.41^{a}±0.16	15.38^{a}±0.17	16.73^{b}±0.14
4	15.21^{a}±0.11	15.64^{b}±0.07	15.69^{b}±0.09	17.14^{c}±0.06
5	15.50^{a}±0.18	16.01^{a}±0.15	15.91^{a}±0.07	17.56^{b}±0.19
6	15.73^{a}±0.21	16.26^{a}±0.11	16.13^{a}±0.09	18.34^{b}±0.18
7	16.06^{a}±0.1	16.66^{b}±0.09	16.40^{b}±0.07	18.75^{c}±0.08
8	16.15^{a}±0.22	17.06^{b}±0.24	16.76^{b}±0.02	19.50^{c}±0.05
9	16.98^{a}±0.20	17.98^{b}±0.13	17.43^{ab}±0.21	20.41^{c}±0.08
10	17.56^{a}±0.18	18.67^{b}±0.07	18.19^{ab}±0.31	21.50^{c}±0.28
11	18.42^{a}±0.13	19.20^{b}±0.25	19.54^{b}±0.19	22.70^{c}±0.06
12	19.12^{a}±0.05	19.97^{b}±0.11	21.27^{c}±0.12	24.30^{d}±0.18

($p \leq 0.05$) when compared to the cumulative weight gain of first (2.23 g) and second (2.36 g) months of fish injected with 1.0 µg g^{-1} body weight of the hormone.

Discussion

Enhancing growth of farmed fish using recombinant growth hormone can be achieved by different methods of delivery *viz*., injection, diet, osmotic shock and immersion (Funkenstein, 2006). Growth hormone gene in a large variety of fish species has been cloned, expressed and the recombinant protein has been used in aquaculture industry. Among these are the tuna (Kimura, 1991), Indian major carps (Venugopal *et al*., 2002), gold fish (Chan *et al*., 2003), common carp (Li *et al*., 2003), giant catfish (Promdonkoy *et al*., 2004) and flounder (Zang *et al*., 2007). But, the effective utilisation of recombinant fish growth hormone in aquaculture depends on biological activity of the hormone. Biologically active recombinant fish growth hormone has been successfully produced in *E. coli*, *Saccharomyces cerevisiae*, *Pichia pastoris* and the microalgae *Chlorella ellipsoidea* and *Synechocystis* sp. (Zang *et al*., 2007).

In the present work, we have cloned a 550 bp long cDNA encoding mature GH of *P. hypophthalmus* (PhGH) and expressed in *E. coli* host. The size of the recombinant fusion protein (PhGH) produced was about 23 kDa, including the 6X His tag (approx. 1.6 kDa) and hence, the original polypeptide constituting the functional hormone is of about 21.5 kDa consisting of 200 amino acids. The fusion rPhGH was expressed after induction and resulted in accumulation and aggregation as inclusion bodies, which is a common phenomenon for over expressed or bacterial recombinant proteins having disulfide bonds (Sciara *et al*., 2006). Thus, to isolate the protein from inclusion bodies and to produce native conformation, the strategy of denaturaion, renaturation and folding was used. After renaturation and folding, the total estimated concentration of the protein was about 20 mg l^{-1} of *E. coli* culture. The concentration of the expressed protein is lower than the GH expressed from several fishes, including 150 mg l^{-1} of *Pangasianodon gigas* rGH in *E. coli* (Promdonkoy *et al*., 2004), 200 mg l^{-1} of carp and 100 mg l^{-1} of *Lateolabrox japonicus* growth hormone in *P. pastoris* (Caelers *et al*., 2005). However, a proportionately low level expression of recombinant protein to cellular protein in *E. coli* cells was also reported in eel (5%), rainbow trout (7.7%), chum salmon (15%) and tilapia (20%) (Tsai *et al*., 1995). The observed lower protein concentrations in the study might be due to the formation of insoluble inclusion bodies and loss during purification.

The *in vivo* assay for testing the biological activity of rPhGH indicated that the fish injected with 1.0 µg g^{-1} body weight (T3) grew faster than the control and other two treatment groups, injected with 0.25 and 0.5 µg g^{-1} body weight (T1 and T2). Overall growth performance among the treatments showed significant difference ($p<0.05$), with weight gain in T3 being significantly higher ($p<0.05$) than that of T1, T2 and control group. This significant difference was observed after two weeks of injection, which implies that the growth hormone would not promote the growth directly but may require other intermediate factors to introduce its functions. In accordance with our study, biological activity of *Cyprinus carpio* (Fine *et al*., 1993) and *Paralichthys olivaceus* (Zang *et al*., 2007) growth hormones showed significantly higher growth differences between control and treatments. On the contrary, Promdonkoy *et al.* (2004) observed that administration of 0.1 µg of *P. gigas* GH showed faster growth than control but the difference was not statistically significant.

In the present study, growth stimulating effect of rPhGH on rohu (*L. rohita*) fingerlings was observed, which is in agreement with the results of several authors *viz*., Sugimoto *et al*. (1990); Fine *et al*. (1993); Jeh *et al*. (1998); Promdonkoy *et al*. (2004); Sciara *et al*. (2011) and Linan-Cabello *et al*. (2013), who observed the growth promoting effect of recombinant growth hormone of eel, common carp, flounder, giant catfish, pejerrey and nile tilapia, respectively. Although the recombinant growth hormone of several fishes showed biological activity, the optimum concentration required to enhance the growth varies among the fish species. Therefore, in the present study, three different concentrations were studied to know the optimum concentration of rPhGH for stimulating growth of fish, and it was observed that the weight gain of fish treated with 1 µg g^{-1} was higher than the weight gain when treated with 0.25 and 0.5 µg g^{-1} body weight. Therefore, the results suggest that high concentration of rPhGH is required for stimulating the biological activity. Similarly, the requirement of higher dose of recombinant growth hormones were also observed by Wilson *et al*. (1988), who reported that fish which received 10 µg g^{-1} of recombinant growth hormone showed faster growth. Li *et al*. (2003) observed the weight of tilapia injected with 1 µg g^{-1} of growth hormone was higher than the fish injected with 0.1 µg g^{-1} and similarly, cinnamon clownfish injected with 1 µg g^{-1} body weight of rGH showed increased body length, when compared to 0.1 µg g^{-1} of hormone (Kim *et al.,* 2014). However, some of the earlier studies also observed that higher dose of recombinant growth hormone treatments have less effect on growth enhancement. Adverse effect caused by higher dose of rGH were reported by Tsi *et al*. (1995), who observed that the weight gain of fish treated with 1 µg g^{-1} was less than that of the fish treated with 0.1 µg g^{-1}. Promdonkoy *et al*. (2004) observed that the optimum level of catfish GH to increase growth of gold fish should be less than 1 µg g^{-1} of fish body weight. Results of different studies indicate that the growth promoting potential of recombinant fish growth hormone varies among different fish species. However, in addition to the species specific action, the growth promoting effect of recombinant hormone may also be determined by fish growth physiology, binding capacity between GH and growth hormone receptors and potency of the hormone. Results of the present study has shown that the biologically active recombinant catfish growth hormone can be efficiently produced using pET-32a(+) expression vector in *E. coli,* and it promoted growth of rohu fingerlings at the concentration of 1 µg g^{-1} of fish body weight. Many investigators have demonstrated that the administration of exogenous recombinant growth hormone as a feed supplement could be a viable method to enhance growth rate of cultured fish. However, limitations such as

formation of inclusion bodies and need for purification of proteins by a complicated procedure before use restrict its wider application (Friesh and Reardon, 1993). Use of appropriate vectors for extracellular expression of recombinant growth hormone in a probiotic bacterium may help to avoid the complicated purification process. Further, use of nanotechnological approaches for efficient delivery of exogenously produced recombinant fish GH may help in effective use of the protein for enhancing fish growth in commercial aquaculture.

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